TAMARIND SUPPLEMENTATION AMELIORATES FLUORIDE-INDUCED GLUCOSE INTOLERANCE AND INSULIN RESISTANCE IN RATS

Vakdevi Validandi,[a] Shankar Rao Gourineni,[a] Srinivas Dheeravath,[a] Balakrishna Nagalla,[b] Arjun L Khandare[a,*]

Hyderabad, India

ABSTRACT: In addition to causing dental and skeletal fluorosis, exposure to high levels of the fluoride ion (F) can alter glucose homeostasis and lead to insulin resistance (IR). Tamarind can act as an antidiabetic agent and the present study was planned to investigate (i) the effect of F on IR and (ii) the protective effect of tamarind (*Tamarindus indica*) fruit extract (TFE) supplementation on F-induced glucose intolerance and IR. For this purpose, Wistar National Institute of Nutrition (NIN) rats (n=18) were taken and randomly distributed into three groups: group I (control) were treated with normal water, group II (F) were treated with 100 ppm F water, and group III (F+TFE) were treated with 100 ppm F water + TFE (200 mg/kg body weight). After 6 months, an oral glucose tolerance test (OGTT) and the insulin profile were studied. The results of the study revealed that the homeostasis model assessment for insulin resistance (HOMA-IR) was unaffected by F but in the F+TFE treated group it was significantly decreased. A significant increase in the plasma glucose levels was observed in the F group at 30, 60, and 120 min ($p<0.05$, $p<0.001$, $p<0.05$, respectively), compared to both the control and the F+TFE groups. The plasma insulin levels were significantly higher ($p<0.05$) in the F group compared to the control group at 30 and 60 min during the OGTT. In the F+TFE group, with tamarind supplementation, the plasma insulin levels were (i) significantly lower ($p<0.05$) than in the F group, and (ii) were significantly higher ($p<0.05$) than in the control group at 30 min but not at 60 min. In the F group, the area under the curve (AUC) for glucose and insulin was significantly higher ($p<0.05$) as compared to the controls, while the F+TFE treatment restored the AUC for glucose and insulin to the control levels. The ratio of AUC glucose to AUC insulin was significantly lower ($p<0.05$) in both the F-treated and the F+TFE-treated rats compared to the control. In conclusion, the TFE supplementation ameliorated glucose intolerance and insulin resistance induced by F treatment in rats.

Keywords: Fluoride; Glucose intolerance; HOMA-IR; Insulin resistance; Tamarind fruit extract.

INTRODUCTION

In India, the groundwater or drinking water sources of 23 of the 36 states and union territories are contaminated with the fluoride ion (F) in varying concentrations. Of these, 17 states have F beyond the threshold level of 1.0 ppm in drinking water.[1] The excessive consumption of F not only causes varying degrees of irreversible damage in teeth and bones[2-7] but also has various metabolic and biochemical effects including altered glycolysis, decreased insulin secretion, hyperglycemia, and the development of insulin resistance (IR).[8-10] An increased F load has been shown to induce changes in insulin and glucose levels in normal

[a]Food Toxicology Division, National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India; [b]Department of Statistics, National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India; *For correspondence: Dr Arjun L Khandare, Scientist F and Head, Food Toxicology Division, National Institute of Nutrition, Indian Council of Medical Research, Jamai Osmania PO, Tarnaka, Hyderabad 500 007, India; Fax No: 040-27019074; E-mail: hodfoodtoxicology@gmail.com; alkhandare@yahoo.com.

experimental animals.[11,12] Patients with impaired glucose tolerance had significantly higher fasting serum immunoreactive insulin, higher fasting serum F, and a significantly lower fasting glucose to insulin ratio than that in patients with normal glucose tolerance or control subjects.[13] In earlier studies it was concluded that tamarind *(Tamarindus indica)* can act as an anti-diabetic agent in experimental animals[14,15]and can also ameliorate F-induced toxic effects by enhancing the excretion of urinary F.[16,17] Whether tamarind acts as an anti-diabetic agent or not in F-induced insulin resistance has not been studied so far. Therefore, in the present study it was planned to examine whether or not tamarind fruit extract (TFE) decreases F-induced insulin resistance.

MATERIALS AND METHODS

Preparation of tamarind pulp extract: The *Tamarindus indica* (tamarind) plant material was identified and authenticated by the Department of Botany, University College of Science, Osmania University, India (voucher No. 0238) where the voucher reference specimens were deposited. The TFE was prepared as described in earlier studies.[18]

Animals and treatment: Eighteen, one-month-old male Wistar National Institute of Nutrition (NIN) rats weighing 77.1±12.65 g (mean±SD) were obtained from the National Centre for Laboratory Animal Sciences of the National Institute of Nutrition, Hyderabad, India, were randomly distributed into 3 groups of 6 animals each, and were fed according to the feeding schedules given in Table 1.

Table 1. Distribution of animals and feeding schedule for the different groups

Group	No. of animals	Details of feeding
Control (C)	6	Control pellet diet + normal water (*ad libitum*)
Fluoride (F)	6	Control pellet diet + 100 ppm F water (*ad libitum*)
Fluoride (F) +tamarind fruit extract (TFE)	6	Control pellet diet + 100 ppm F water + 200 mg TFE/kg bodyweight (*ad libitum*)

All the animals were housed individually in stainless steel cages in a temperature and humidity controlled room with a 12 hr light and dark cycle. The animal care and experimental protocols were followed as given in the guidelines approved by the Institutional Animal Ethics Committee (No.P9F/IAEC/2014/II/ALK/WNIN-46M).

Dietary and water intake, body weight, and urinary F: The dietary and water intakes, the body weights, and the urinary F were assessed according to the protocols used in an earlier study conducted at our Institute.[19]

Oral glucose tolerance test (OGTT): OGTT was assessed in the rats at 6 months according to the method reported in earlier studies.[20] Blood samples were collected from orbital sinus flux at 0, 30, 60, and 120 min and plasma was

separated by centrifugation at 3000 rpm for determining glucose and insulin concentrations.

Estimation of glucose and insulin in plasma: Plasma glucose was measured by the glucose oxidase-peroxidase (GOD-POD) method with a kit (Biosystems Diagnostics Pvt Ltd, Tamil Nadu, India) and plasma insulin by a RIA kit (BRIT-DAE, Mumbai, India).

Homeostasis model assessment for insulin resistance (HOMA-IR): Insulin resistance was assessed by the homeostasis model assessment (HOMA–IR) as described earlier for rats[21,22] using the equation:

$$\text{HOMA-IR} = \frac{\text{Fasting plasma glucose (mg/dL)} \times \text{Fasting plasma insulin (µU/mL)}}{2{,}430}$$

To assess the animal's insulin response to a challenge of glucose, the area under the curve (AUC) for glucose and insulin during the OGTT was computed. The AUC for glucose and insulin during OGTT at 0, 30, 60, and 120 min were calculated according to the formulae:

AUC for glucose (mmol/L/hr) = 0.25 × plasma glucose at 0 min (mmol/L)
+ 0.5 × plasma glucose at 30 min (mmol/L)
+ 0.75 × plasma glucose at 60 min (mmol/L)
+ 0.5 × plasma glucose at 120 min (mmol/L)

AUC for insulin (µU/mL/hr) = 0.25 × plasma insulin at 0 min (µU/mL)
+ 0.5 × plasma insulin at 30 min (µU/mL)
+ 0.75 × plasma insulin at 60 min (µU/mL)
+ 0.5 × plasma insulin at 120 min (µU/mL).

AUC glucose:AUC insulin ratio: The AUC glucose:AUC insulin ratio was calculated from the values of the AUC for glucose and insulin obtained.

RESULTS

Diet intake, water intake, and body weight: The dietary intake, the water intake, and the body weights of the rats at 2, 4, and 6 months of all the groups are depicted in Tables 2A, 2B and 2C, respectively.

Table 2A. The dietary intake (g) in the different groups at 2, 4, and 6 months.
(Results are expressed as mean±SD; n= 6 rats/group;
TFE=Tamarind fruit extract)

Group	Dietary intake (g)		
	2 months	4 months	6 months
Control (C)	20.542±0.573	17.276±2.12	20.083±1.854
Fluoride (F)	11.470±0.154 [a]*	13.051±0.418 [a]*	17.280±2.675 [a]*
F+TFE	17.160±1.88 [a]*,[b]*	15.933±1.44 [a]*,[b]*	19.206±1.224 [a]*

[a]: compared to control group: *p<0.05; [b]: compared to F group: *p<0.05.

Table 2B. The water intake (mL) in the different groups at 2, 4, and 6 months.
(Results are expressed as mean±SD; n= 6 rats/group;
TFE=Tamarind fruit extract)

Group	Water intake (mL)		
	2 months	4 months	6 months
Control (C)	42.613±3.53	44.496±3.08	43.330±4.798
Fluoride (F)	21.521±1.94[a]*	26.613±1.52[a]*	30.426±0.248[a]*
F+TFE	22.430±1.00[a]*	26.423±1.93[a]*	29.806±2.397[a]*

[a]: compared to control group: *p<0.05.

Table 2C. Body weights (g) in the different groups at 2, 4, and 6 months.
(Results are expressed as mean±SD; n= 6 rats/group;
TFE=Tamarind fruit extract)

Group	Body weights (g)		
	2 months	4 months	6 months
Control (C)	189.81±17.374	317.300±23.014	448.166±44.382
Fluoride (F)	113.360±14.237[a]*	255.510±12.517[a]*	332.833±59.509[a]*
F+TFE	136.632±30.560[a]*,[b]*	273.166±26.984[a]*	388.661±50.29[a]*,[b]*

[a]: compared to control group: *p<0.05; [b]: compared to F group: *p<0.05.

Urinary F: The urinary F levels among all the groups at 2, 4, and 6 months are given in Table 3.

Table 3. Urinary F levels (mg/24 hr) in the different groups at 2, 4, and 6 months. (Results are expressed as mean±SD; n= 6 rats/group; TFE=Tamarind fruit extract)

Group	Urinary F levels (mg/24 hr)		
	2 months	4 months	6 months
Control (C)	0.041± 0.007	0.065 ± 0.014	0.094 ± 0.011
Fluoride (F)	0.645 ± 0.16[a]*	0.709 ± 0.134[a]*	0.736 ± 0.104[a]*
F+TFE	0.940 ± 0.201[a]*,[b]*	1.048 ± 0.258[a]*,[b]*	1.017± 0.172[a]*,[b]*

[a]: compared to control group: *p<0.05; [b]: compared to F group: *p<0.05.

Fasting plasma glucose and insulin: The fasting plasma glucose was unaffected by the F and the F+TFE treatments as compared to the control group (Figure. 1). The results the HOMA-IR and the AUC glucose: AUC insulin ratio are given in Table 4.

Table 4. HOMA-IR and AUC glucose:AUC insulin ratio in the different groups at 2, 4, and 6 months. (Results are expressed as mean±SD; n= 6 rats/group; HOMA-IR=homeostasis model assessment for insulin resistance; AUC= area under the curve; TFE=Tamarind fruit extract,)

	HOMA-IR	AUC glucose:AUC insulin ratio
Control (C)	1.042±0.316	0.152±0.129
Fluoride (F)	0.858±0.178	0.12±0.006[a]*
F+TFE	0.7068±0.120[a]*	0.141±0.008[a]*,[b]*

[a]: compared to control group: *p<0.05; [b]: compared to F group: *p<0.05.

Glucose response during the oral glucose tolerance test (OGTT): The plasma glucose levels in the F group were significantly higher at 30 ($p<0.05$), 60 ($p<0.001$), and 120 min ($p<0.05$) as compared to the control and F+TFE groups (Figure 1).

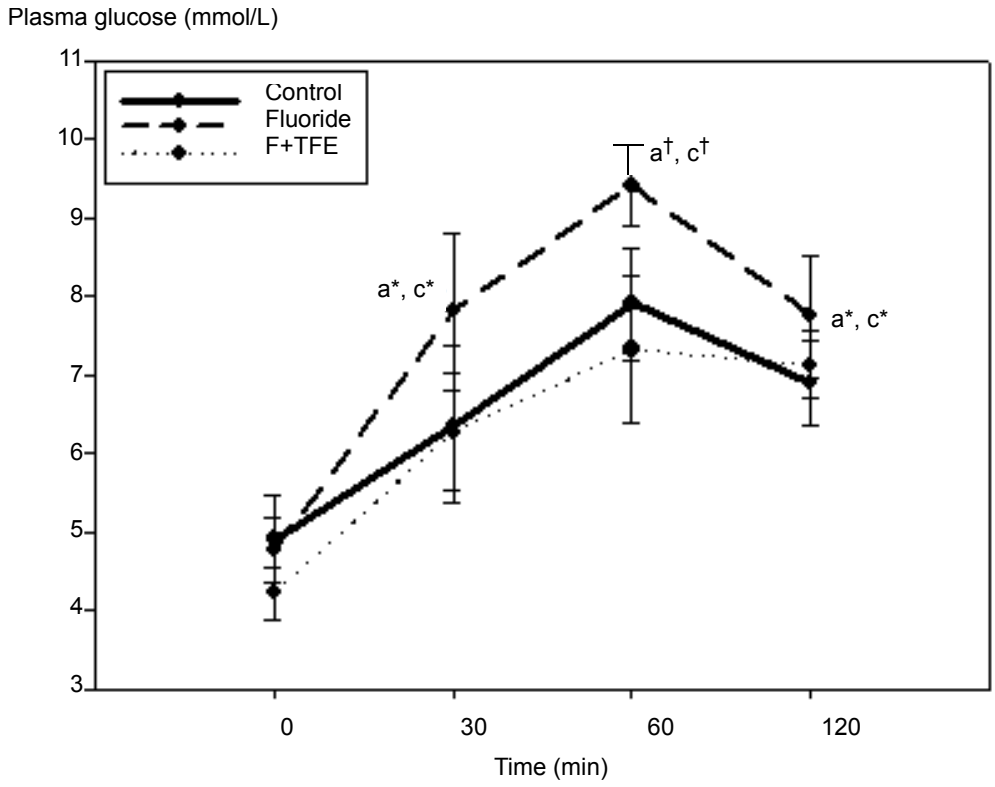

Figure 1. Plasma glucose response during the oral glucose tolerance test in the different groups at 6 months. a: compared to the control group: *p<0.05, †p<0.001; c: compared to the F+TFE group: *p<0.05, †p<0.001. The results are expressed as mean±SD; n= 6 animals/group.

Insulin response during OGTT: In the F group, in comparison to the control group, there was a significant increase in the plasma insulin levels ($p<0.05$) at both 30 and 60 min (Table 5).

Table 5. Insulin response during oral glucose tolerance test in the different groups at 2, 4, and 6 months. (Results are expressed as mean±SD; n= 6 rats/group; TFE=Tamarind fruit extract)

Group	Insulin (µU/mL)			
	0 (fasting)	30 min	60 min	120 min
Control (C)	28.17±5.913	42.67±6.121	54.17±5.87	43.00±4.099
Fluoride (F)	24.17±4.119	87.33±8.238[a*]	82.00±6.92[a*,c*]	41.00±6.986
F+TFE	22.67±4.227	59.17±5.87[a*,b*]	49.67±7.737	42.83±3.488

[a]: compared to control group: *p<0.05; [b]: compared to F group: *p<0.05;
[c]: compared to F+TFE group: *p<0.05.

In the F+TFE group, compared to the F group, the plasma insulin levels were significantly lower ($p<0.05$) at both 30 and 60 min (Table 5). In the F+TFE group, compared to the control group, the plasma insulin levels were significantly increased ($p<0.05$) at 30 min but not at 60 min (Table 5). Interestingly, at all three time points, 30, 60, and 120 min, during the OGTT, the F+TFE treatment significantly decreased the plasma glucose levels compared to the F group (Figure 1).

Furthermore, the area under curve (AUC) for both glucose and insulin was higher in the F group compared to the control and F+TFE groups ($p<0.05$) (Figure 2).

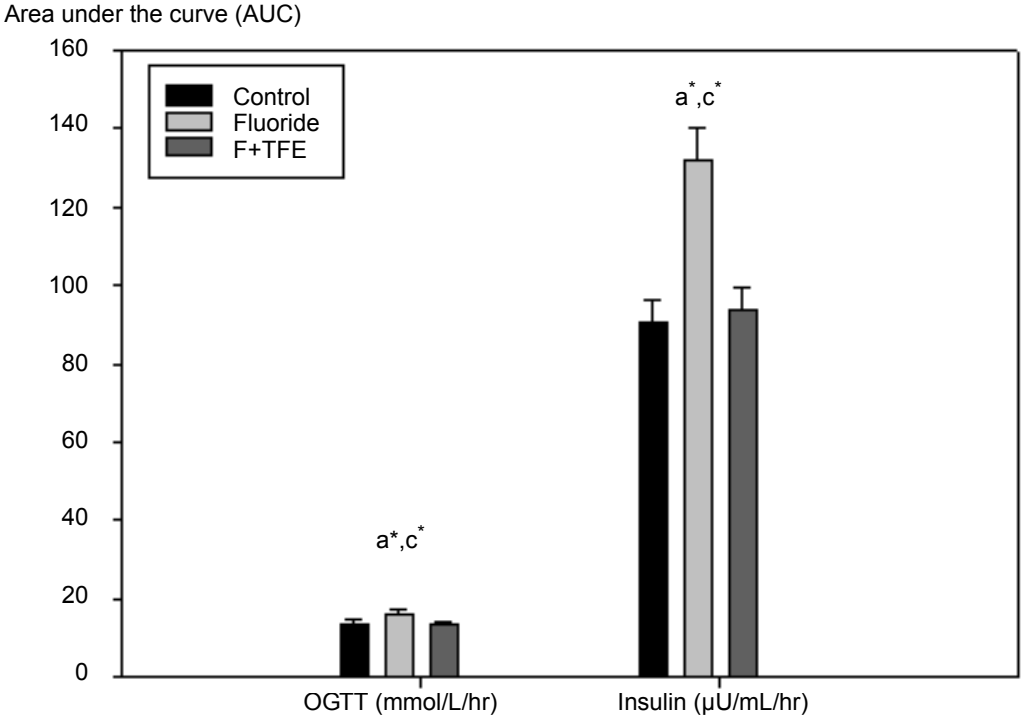

Figure 2. Area under the curve (AUC) for glucose and insulin during the oral glucose tolerance test in the different groups at 6 months. a: compared to the control group: *p<0.05, c: compared to the F+TFE group: *p<0.05. The results are expressed as mean±SD; n= 6 animals/group.

The ratio of AUC glucose to AUC insulin was significantly lower ($p<0.05$) in the F group compared to the control and F+TFE groups (Table 4).

DISCUSSION

In the present study, in the F group, compared to control group, the significant decreases ($p<0.05$) in the dietary and water intake, and in the body weight corroborates the earlier studies.[23-25] However, in the F+TFE group, compared to the F group, there were significant increases ($p<0.05$) in the dietary intake at 2 and 4 months and the body weight at 2 and 6 months which indicates that the tamarind supplementation had a beneficial effect. The enhanced excretion of urinary F in

the F+TFE group compared to the F group at 2, 4, and 6 months in the present study corroborates the earlier studies conducted in our laboratory.[16,17] Earlier studies conducted in human volunteers and in experimental animals revealed that F ingestion increases the plasma F and decreases the plasma insulin with an accompanying increase in serum glucose levels.[11] According to an earlier study,[26] above 5μM of F in plasma disrupts glucose homeostasis. Previous studies reported that in rats fed with 100 ppm F in their drinking water, the serum F levels increased to 5–10 μM.[27,28] Similarly, in the present study, the administration of 100 ppm F through drinking water to the F groups might have increased the serum F levels to 5–10 μM and disrupted the glucose metabolism. However, the serum F levels were not estimated in the present study.

Earlier studies also indicate that the enzymes in the glycolytic pathway, such as hexokinase, enolase, and pyruvate kinase, are all subject to F inhibition.[29] The present findings are also similar to the results of earlier studies[11,13] In the present study, the rats in the F-treated group showed impaired glucose metabolism, with a significant increase, compared to the control group, in the plasma glucose levels at 30, 60, and 120 min during a OGTT. In the F group, there was a delay in the glucose clearance, as evidenced by the high plasma glucose levels at 120 min compared to the other groups, even though the plasma insulin levels during the OGTT were significantly higher in the F group, compared to both the control and F+TFE groups at 30 and 60 min. However, the rats were not able to maintain normal glucose levels, thus indicating the condition of insulin resistance in the experimental animals as reported in earlier studies.[8,10-13] The plasma glucose levels in the F+TFE group at 30, 60, and 120 min were not significantly different to the values in the control group. The plasma insulin level was increased in the F+TFE group, compared to the control group, at 30 min but not at 60 min. This suggests that tamarind has an ameliorative potential in F-induced insulin resistance in rats. In the present study, tamarind also acts as an anti-diabetic agent as reported in earlier studies.[14,15] The presence of elevated plasma insulin levels alongside elevated plasma glucose in the F group indicate either the secretion of bio-inactive insulin or the presence of insulin resistance as observed in earlier studies.[13] The effect of F on insulin sensitivity described in this study could be the consequence of an interaction between F and the pancreas.[30-32]

An increase in AUC for glucose and insulin compared to controls indicates the presence of insulin resistance in rats.[20] In the present study, the AUC for glucose and insulin were significantly higher ($p<0.05$) in the F group in comparison to the control group indicating insulin resistance in the rats. However, in the F+TFE group the AUC for glucose and insulin were restored to the control levels thus indicating that the tamarind fruit extract had a protective effect. This protective role of tamarind in the F-induced glucose intolerance and insulin resistance in rats may be due to presence of proanthocyanidins, one of the components present in TFE.[34,35]

CONCLUSION

Tamarind fruit extract supplementation ameliorates F-induced glucose intolerance and insulin resistance in F-intoxicated rats. However, the present study is at only a preliminary level and more studies are recommended to confirm the findings. Nonetheless, the present findings significantly add to the existing knowledge on the amelioration of F-induced toxicosis.

ACKNOWLEDGEMENTS

We are thankful to the Director-General, Indian Council Medical Research, New Delhi, for financial assistance and to the Director-in-Charge, National Institute of Nutrition, Hyderabad, India, for the provision of research facilities.

REFERENCES

1 Choubisa SL. A brief and critical review on hydrofluorosis in diverse species of domestic animals in India. Environ Geochem Health 2017; doi 10/1007/s 10653-017-9913-x, pp 1-16.
2 Choubisa SL, Choubisa DK, Joshi SC, Choubisa L. Fluorosis in some tribal villages of Dungarpur district of Rajasthan, India. Fluoride 1997;30(4):223-8.
3 Choubisa SL. Endemic fluorosis in southern Rajasthan, India. Fluoride 2001;34(1):61-70.
4 Choubisa SL, Choubisa L, Choubisa D. Osteo-dental fluorosis in relation to nutritional status, living habits, and occupation in rural tribal areas of Rajasthan, India. Fluoride 2009;42(3):210-5.
5 Choubisa SL. Osteo-dental fluorosis in horses and donkeys of Rajasthan, India. Fluoride 2010;43(1):5-10.
6 Choubisa SL. Fluorosis in dromedary camels in Rajasthan, India. Fluoride 2010;43(3):194-9.
7 Choubisa SL. Fluoride toxicosis in immature herbivorous domestic animals living in low fluoride water endemic areas of Rajasthan, India: an observational survey. Fluoride 2013;46(1):19-24.
8 Menoyo I, Puche RC, Rigalli A. Fluoride-induced resistance to insulin in the rat. Fluoride 2008;41(4):260-9.
9 Eliud A, Garcia-Montalvo, Hugo Reyes-Perez, Luz M. Del Razo. Fluoride exposure impairs glucose tolerance via decreased insulin expression and oxidative stress. Toxicology 2009; 263:75-83.
10 Chiba FY, Colombo NH, Shirakashi DJ, Da Silva VC, Moimaz SAS, Garbin CAS, et al. NaF treatment increases TNF-α and resistin concentrations and reduces insulin signal in rats. J Fluorine Chem 2012;136:3-7.
11 Rigalli A, Ballina JC, Roveri E, Puche RC. Inhibitory effect of fluoride on the secretion of insulin. Calcif Tissue Int 1990;46(5):333-8.
12 Rigalli A, Ballina JC, Puche RC. Bone mass increase and glucose tolerance in rats chronically treated with sodium fluoride. Bone and Miner 1992;16(2):1018.
13 Trivedi N, Mithal A, Gupta SK, Godbole MM. Reversible impairment of glucose tolerance in patients with endemic fluorosis. Fluoride Collaborative Study Group. Diabetologia1993;36(9):826-8.
14 Rajkumar Maiti, Uttam Kumar Das, Debidas Ghosh. Attenuation of hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats by aqueous extract of seed of *Tamarindus indica*.Biol Pharm Bull 2005;28(7):1172-6.
15 Yerima M, Anuka JA, Salawu OA, Abdu-Aguye I. Antihyperglycaemic activity of the stem-bark extract of *Tamarindus indica* L. on experimentally induced hyperglycaemic and normoglycaemic Wistar rats. Pak J Biol Sci 2014;17(3):414-8.

16 Khandare AL, Kumar PU, Lakshmaiah N. Beneficial effect of tamarind ingestion on fluoride toxicity in dogs. Fluoride 2000;33(1):33-8.
17 Khandare AL, Rao GS, Lakshmaiah N. Effect of tamarind ingestion on fluoride excretion in humans. Eur J Clin Nutr 2002;56(1):82-5.
18 Gupta AR, Dey S, Saini M, Swarup D. Protective effect of *Tamarindus indica* fruit pulp extract on collagen content and oxidative stress induced by sodium fluoride in the liver and kidney of rats. Toxicol Environ Chem 2013;95:1611-34.
19 Shankar P, Ghosh S, Bhaskarachary K, Venkaiah K, Khandare AL. Amelioration of chronic fluoride toxicity by calcium and fluoride-free water in rats. Br J Nutr2013;110(1):95-104.
20 Suryanaryana P, Madhusoodan AP, Bhanuprakash GB. Insulin resistance mediated biochemical alterations in eye lens of neonatal streptozotocin-induced diabetic rat. Indian J Exp Biol 2011;49:749-55.
21 Mather K. Surrogate measures of insulin resistance: of rats, mice and men. Am J Physiol Endocrin Metab 2009;296(2):E398.
22 Cacho J, Sevillano J, de Casro J, Herrera E, Ramos MP. Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. Am J Physiol Endocrin Metab 2008;295(5):E1269.
23 Coetze CB, Casey NH, Meyer JA. Fluoride tolerance of laying hens. Br Poult Sci 1997;38:597-602.
24 Dunipace AJ, Edward JB, Wilson ME, Zhang W, Katz BP, Stookey GK. Chronic fluoride exposure does not cause detrimental, extra skeletal effects in nutritionally deficient rats. J Nutr 1998;128:1392-400.
25 Frank A, Anke M, Danielsson R. Experimental copper and chromium deficiency and additional molybdenum supplementation in goats I. Feed consumption and weight development. Sci Total Environ 2000;249:133-42.
26 de la Sota M, Puche RC, Rigalli A, Fernandez LM, Benassati S, Boland R Changes in bone mass and in glucose homeostasis in subjects with high spontaneous fluoride intake. Medicina (B Aires) 1997;579(4):417-20.
27 Den Besten PK, Yan Y, Featherstone JDB, Hilton JF, Smith CE, Li W. Effects of fluoride on rat dental enamel matrix proteinases. Arch Oral Biol 2002;47:763-70.
28 Den Besten P, Wu Li. Chronic Fluoride Toxicity: Dental Fluorosis. Monogr Oral Sci. 2011; 22:81-96.
29 Adamek E, Pawłowska-Goral K, Bober K. *In vitro* and *in vivo* effects of fluoride ions on enzyme activity. Ann Acad Med Stetin 2005;51(2):69-85.
30 Dabrowska E, Balunowska M, Letko R, Szynaka B. Ultrastructural study of the mitochondria in the submandibular gland, the pancreas and the liver of young rats, exposed to NaF in drinking water. Rocz Akad Med Bialymst 2004;1:180-1.
31 Menoyo I, Rigalli A, Puche RC. Effect of fluoride on the secretion of insulin in the rat. Arzneimittel-Forschung 2005;55(8):455-60.
32 Stawiarska-Pieta B, Paszczela A, Grucka-Mamczar E, Szaflarska-Stojko E, Birkner E. The effect of antioxidative vitamins A and E and coenzyme Q on the morphological picture of the lungs and pancreata of rats intoxicated with sodium fluoride. Food Chem Toxicol 2009;47(10):2544-50.
33 Pinar K. *Tamarindus indica* and its health related effects. Asian Pac J Trop Biomed 2014; 4(9):676-81.
34 Jiao L, Zhang X, Huang L, Gong H, Cheng B, Sun Y, et al. Proanthocyanidins are the major anti-diabetic components of cinnamon water extract. Food Chem Toxicol2013;56:398-405.
35 Uttara S, Sadhana S, Anita K. Therapeutic potential of anti-diabetic nutraceuticals. Phytopharmacology 2012;2(1):144-69.